Treadmill Exercise Prevents Recognition Memory Impairment in VD rat model through Enhancement of Hippocampal Structural Synaptic Plasticity

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Abstract

In vascular dementia (VD), memory impairment caused by the damage of synaptic plasticity is the most prominent feature that afflicts patients and their families. Treadmill exercise has proven beneficial for memory by enhancing synaptic plasticity in animal models including stroke, dementia, and mental disorders. The aim of this study was to examine the effects of treadmill exercise on recognition memory, and structural synaptic plasticity in VD rat model. Here, our study demonstrated that VD rat exhibited significantly recognition impairment, while treadmill exercise improved recognition memory in VD rat. To further investigate potential mechanisms for the treadmill exercise-induced improvement of recognition memory, we examined hippocampal structural synaptic plasticity by means of transmission electron microscopy and golgi staining in VD rat that had undergone 4 weeks of treadmill exercise. The results demonstrated that VD rat causes the damage of structural synaptic plasticity. However, treadmill exercise led to increases in synapse numbers and the number of dendritic spines in VD rat. Together, the improvement of VD-associated recognition memory by treadmill exercises is associated with enhanced structural synaptic plasticity in VD rat model.

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Abstract:

In vascular dementia (VD), memory impairment caused by the damage of synaptic plasticity is the most prominent feature that afflicts patients and their families. Treadmill exercise has proven beneficial for memory by enhancing synaptic plasticity in animal models including stroke, dementia, and mental disorders. The aim of this study was to examine the effects of treadmill exercise on recognition memory, and structural synaptic plasticity in VD rat model. Here, our study demonstrated that VD rat exhibited significantly recognition impairment, while treadmill exercise improved recognition memory in VD rat. To further investigate potential mechanisms for the treadmill exercise-induced improvement of recognition memory, we examined hippocampal structural synaptic plasticity by means of transmission electron microscopy and golgi staining in VD rat that had undergone 4 weeks of treadmill exercise. The results demonstrated that VD rat causes the damage of structural synaptic plasticity. However, treadmill exercise led to increases in synapse numbers and the number of dendritic spines in VD rat. Together, the improvement of VD-associated recognition memory by treadmill exercises is associated with enhanced structural synaptic plasticity in VD rat model.

Keywords: Treadmill exercise; VD; Recognition memory; Structural synaptic plasticity.

Introduction

Vascular dementia (VD) is considered to be the second most common form of dementia after alzheimer's disease (AD) and accounts for at least 20% of dementia cases[1-2]. VD may be caused by cerebrovascular disease, including ischemic or hemorrhagic stroke, and hypoperfusion ischemic brain injury due to cardiovascular and circulatory disorders[3]. The persistent and irreversible memory impairment in VD patients leads to a serious deterioration in quality of life and places a heavy economic burden on the families of patients[4-5]. Together with the increasing age of the population and improved survival rates from cardiovascular diseases, VD may affect more individuals in the future[6]. Therefore, prevention and treatment of VD is increasingly important at home and abroad, especially in countries with aging populations. It was mentioned as early as 1980's that VD at present may be more amenable to prevention and treatment than AD[7]. Until now, many more drugs exerts memory protective effects in VD patients, including donepezil and tanzhi granules, targeted the amelioration of cognitive impairment by inhibiting neuroinflammation and acetylcholinesterase[8]. But the drug's side effects can include drug dependence and depression.

As a non-pharmacological treatment, physical exercise has proven beneficial for support brain health and function, including but not limited to dampen brain inflammation[9], reducing neuroinflammation[10], and the redistribution of blood flow and neural activity[11]. Among new therapeutic strategies being pursued to minimize cognitive damage, clinical studies have confirmed that physical exercise is associated with lower incidence of vascular dementia[12]. Moreover, various animal models have demonstrated that regular volunteer running or treadmill exercise all can improve memory function in VD rats, in association with protected the function of astrocytes[13-14]. However, the molecular mechanism for exercise-improved memory in VD model remains poorly understood and still need for further study.

The brain changes throughout life at synaptic levels, including morphological and physiological changes. Structural synaptic plasticity is relative with synaptic morphology, which is thought to underlie higher cognitive processes such as memory storage and recall[15]. The role of structural synaptic plasticity between physical exercise and memory function has been recognized on both animal models and humans. Strong clinical and experimental evidence support that during exercise, modifications in the morphology of synaptic generate structural synaptic plasticity changes that is assumed to underlie enhanced cognitive processes such as memory function[16-17]. Furthermore, in vivo evidence has been provided to illustrate the molecular mechanisms of exercise on memory function possibly via regulating dendritic spine formation[18] and the ultrastructural morphology of synapses[19], in addition to memory recovery in VD[20] and other mental disease[21]. These findings suggest an important role for structural synaptic plasticity in the pathogenesis of VD and it has become an interesting target for therapeutic intervention. However, whether exercise improve memory function is related with structural synaptic plasticity still needs to be further explored. Here, the aim of our present study is to determine the regulatory paradigm of physical exercise on memory function and structural synaptic plasticity in the hippocampus under VD model.

Methods

Experimental animals and grouping

Male Sprague-Dawley rats (8 weeks of age) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd and housed three per cage under normal light-dark cycle with food and water (Temperature: 22 ± 3°C; Humidity: 40 to 70%). All rats were randomly assigned into four groups (n = 6 each): Control group (C group), vascular dementia group (VD group), treadmill exercise and vascular dementia group (Exe-VD group), treadmill exercise and black group (Exe group). Body weight was measured every three days, and other treatments were performed at designated times according to the experimental timeline (Fig. 1).

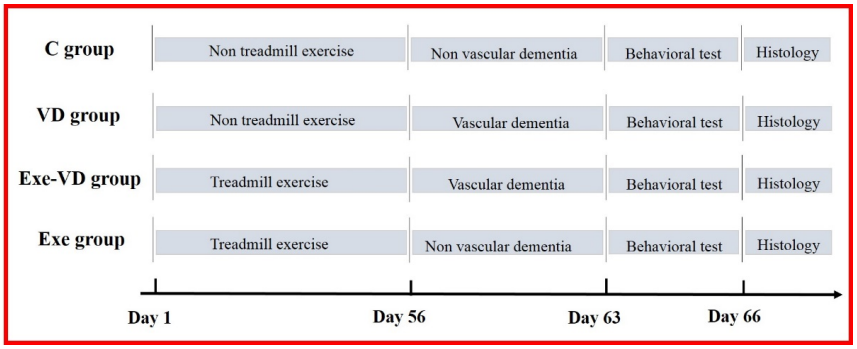

Fig. 1. The experimental timeline.

Treadmill exercise

The treadmill exercise was performed according to previous literature[22]. During the adaptation period, the exercised rats were allocated in the test room for 30 min acclimation, followed by treadmill running for 30 min on 3 consecutive days (first day at 8 m/min; second day at 10/min; third day at 12m/min). Then, those rats were subjected to a treadmill exercise protocol at the speed of 12 m/min for 60 min. In total, rats were trained for 1 h per day, 5 days per week, for a total of 4 weeks.

VD model

VD model was induced by bilateral common carotid arteries operation according to previous reported[23]. First, under deep anesthesia with 10% chloral hydrate (350 mg/kg), bilateral common carotid arteries of rats in VD and Exe-VD groups were identified and isolated from vagus through a ventral midline cervical incision, then tightly ligated using 10-0 suture thread on both ends. The rats in the control group were subjected to the same surgical procedure except that the common carotid arteries were exposed but not

ligated. The "Zea-Longa 5-point scale" was performed according to previously described methods to verify whether the animal model had been successfully established. A score between 1 and 4 indicates successful modeling of VD. Rats not showing neurological defect were excluded from the study.

Table 1 "Zea-Longa" 5-point scale

Point	Symptom
0 point	no neurological deficit
1 point	failure to extend left forepaw fully
2 point	circling to the left
3 point	falling to the left
4 point	no spontaneous walking with depressed level of consciousness
5 point	Death

Behavior test

Behavior test included open filed test and recognition memory test. First day, the experimental apparatus of open filed test was a black open-field box (100cm ×100cm×50cm), positioned in a dimly illuminated room. Each rat were allowed to freely explore 10 min. During the test session, moving distance, time in center area and number of times to enter center area were measured automatically by a computer-based system[24].

Second day, each rat was placed in the black open-field box containing two identical objects and allowed to explore two identical objects (A1 and A2) for 10 minutes. The object with most object interactions were defined as similar object. After 5 minutes or 24h, similar object was replaced with a novel object and the rat were allowed to explore for 10 minutes. A discrimination index was calculated as the difference in number exploring the novel and familiar object, expressed as the ratio of the total number spent exploring both objects. (i.e., [Number Novel / Number Novel + Number Familiar] × 100%). In addition, all object combinations and locations were used in a balanced manner to reduce potential bias due to preferences for specific locations or objects[25].

Transmission Electron Microscopy

Transmission Electron microscopy was performed to observed synaptic ultrastructure in the hippocampus[26]. The sample (hippocampal rat brain tissue) were blocked with fresh TEM fixative for 2 hours and were dehydrated in graded ethanol solutions (30% to 100%). Brain tissues were stained with resin penetration and embedding as well as keep in 37oven overnight, then moved into 65 oven to polymerize for 2 day. Images were captured with a transmission electron microscope (Hitachi, Japan).

Golgi Staining

Golgi staining was performed as previously described. Briefly, the fresh whole brain was obtained and immediately fixed with 4% paraformaldehyde for 48h. Then, the sample was placed in to a 45ml EP tube containing Golgi-cox staining solution for 14 days. After treated with distilled water, 80% glacial acetic acid and 30% sucrose, respectively, the tissue were cut into 100 microns and dry in the dark overnight. The sections were slides with concentrated ammonia water and hardening fixing solution for 15 min, then washing with distilled water for 3 min, dry and seal the section with glycerin gelatin. Images of brain tissue by panoramic scanning with digital slice scanner[27].

Statistics

Data in this experiment are presented as the mean +- SEM. Data sets were compared with two-way ANOVA followed by Tukey's post hoc analysis. Post-hoc analyses were performed only when ANOVA yielded a significant main effect or a significant interaction between the two factors. Results were considered to be significant at $p < 0.05$.

Results

Neurobehavioral assessment of VD model

A neurological evaluation was performed 7 day after 2-VO and scored on a 5-point scale. As shown in Fig. 2, rats in the control group had no neurological deficit. The results also showed that: the grade score were significantly higher in the VD group when compared with C group ($P<0.001$; Fig. 2). In addition, compared with the VD group, the rats in Exe-VD group significantly decrease the grade score ($P<0.05$; Fig. 2). Thus, we concluded that vascular dementia was successfully developed in our experiment undergoing the 2-VO procedure.

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Fig. 2. Neurological defect score in each group.$^{\#\#\#}P<0.001$ vs. C group;* $P<0.05$ vs. VD group.

VD-induced anxiety behavior was ameliorated by treadmill exercise

In open field test, the VD rats had fewer number of times to enter the central area and spent less time in center area than control rats, whereas the total distances traveled within the open field were the same between the control and VD groups ($P<0.01$, $P<0.001$; Fig. 3A-B). After treadmill exercise intervention, significantly elevated number of times to enter the central area and time in center area in the Exe-VD rats when compared with VD group ($P<0.01$, $P<0.001$; Fig. 3A-B), whereas did not differ between the VD and Exe-VD groups. This result suggests that the VD rats have a mild anxiety, while their locomotor activity is normal. In addition, the anxiety behavior in VD rats can be rescued by physical exercise.

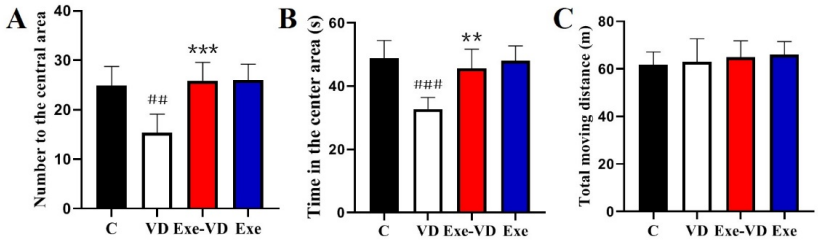

Fig. 3. Treadmill exercise prevented stress-induced anxiety- like behavior in open field test. $^{\#\#}p<0.01$ and$^{\#\#\#}P<0.001$ vs. C group;$^{**}p<0.01$ and $^{***}P<0.05$ vs. VD group.

VD-induced recognition memory impairment was ameliorated by treadmill exercise

As shown in Fig. 4, the discrimination index of each group were obtained by recognition memory test. When compared with control rats, the discrimination index of VD rats was significantly lesser ($P<0.05$, $P<0.01$; Fig. 4B). Conversely, the discrimination index of Exe-VD rats was significantly greater than VD rats ($P<0.05$, $P<0.01$; Fig. 4B). Thus, these findings thus indicate that treadmill exercise rescued recognition memory impairment in VD model.

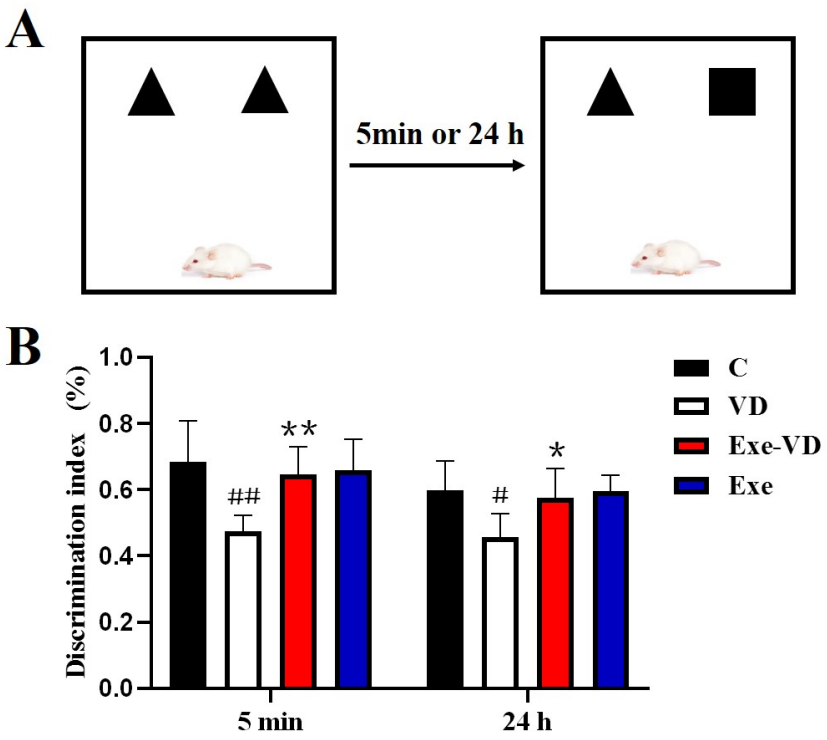

Fig. 4. Treadmill exercise prevents recognition memory impairment in VD rats. $^{\#}p<0.05$ and$^{\#\#}P<0.01$ vs. C group;$^{*}p<0.05$ and $^{**}P<0.01$ vs. VD group.

Treadmill exercise rescued VD-induced synaptic ultrastructure deficits

Synaptic ultrastructure was the basis of structural synaptic plasticity. Thus, we assessed hippocampal synapse numbers by transmission electron microscope that are critical for the transmission of information related to learning and memory. As shown in Fig. 3, hippocampal synapse numbers were significantly decreased in the VD group compared to the control group ($P<0.05$; Fig. 5B). These alterations were reversed by treadmill exercise, and the rats exhibited healthier synaptic ultrastructure, including significantly increased synapses and greater synaptic connection density (($P<0.01$; Fig. 5B)). Meanwhile, in the Exe group, the number of synapses in the hippocampus were significantly increased than those in C group ($P<0.05$; Fig.5B). In conclusion, treadmill exercise ameliorated the above damage and protect the ultrastructure of synapses, suggesting improved structural synaptic plasticity.

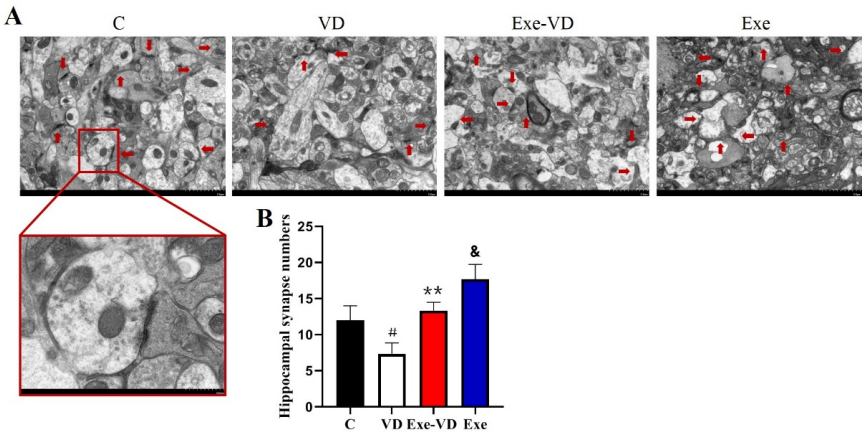

Fig. 5. Treadmill exercise increases synapse numbers of hippocampus in VD rats. A). Representative electron microscope images in each group. B). The number of hippocampal synapse (red arrowhead) in each group. The synapses are marked by the red arrowheads.$^{\#}P<0.05$ and$^{\&}P<0.05$ vs. C group;$^{**}P<0.01$ vs. VD group.

Treadmill exercise rescued dendritic damage of synapse caused by VD.

To further confirm the neuroprotective effect of treadmill exercise on recognition in VD rats was associated with structural synaptic plasticity, we next analyzed spine density. The results demonstrated that the spine numbers of the hippocampus were significantly decreased in the VD group compared to the control group ($P<0.05$; Fig. 6B). Meanwhile, compared with VD group, hippocampal spine numbers were significantly increased in Exe-VD group ($P<0.05$; Fig. 6B). Thus, treadmill exercise blocked a decrease in the spine density of hippocampus in VD rats.

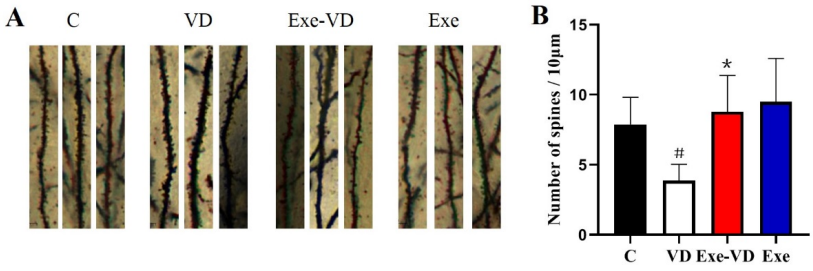

Fig. 6. Exercise rescued spine density caused by VD. A). Representative hippocampus microphotographs of the spines on secondary dendrites of neurons in each group. B). Number of spine per 10μm in each group.$^{\#}P<0.05$ vs. C group;$^{*}P<0.05$ vs. VD group.

Discussion

Our study aimed at understanding the link between exercise and AD have focused on structural synaptic plasticity. In this study, we used recognition memory test coupled with transmission electron microscope and golgi staining methods to track dynamics of structural synaptic in the hippocampus after treadmill exercise on VD rat. We found that the restore of synaptic ultrastructure and spine density in the hippocampus with exercise, in additional to improved recognition memory in VD model. Our findings suggest that strengthening structural synaptic plasticity may represent a potential mechanism by which treadmill exercise prevents impairment of recognition memory in VD model.

In our study, we first found that the discrimination index in VD rats was significantly lesser than control rats when exposed to the novel object 5 minutes and 24 hours after they were familiarized with an identical set of objects. As the disease progresses, patients experience progressive memory loss and emotional disorder in daily life, including communication disorder and anxiety[28-29]. In vivo study also confirms impaired discrimination index in recognition memory test[30], which is one of the most common paradigms to assess hippocampal-dependent memory, including short-term recognition memory and long-term recognition memory[31]. In general, short-term memory only refers to the short-term storage of information, whereas long-term memories are required for remembering information[32]. In addition, we also provided vivo evidence that VD rats have a mild anxiety in open field test. It was consisted with clinical investigations. Thus, we demonstrated that the impairment of recognition memory and anxiety-like behavior in VD progression. Furthermore, our study also revealed that treadmill exercise rescued recognition memory impairment and anxiety-like behavior in VD model in the recognition memory test. This result was consisted with previous studies[33-34]. Taken together, VD rats exhibited impairment recognition memory, and the robust decrease of recognition memory errors under the recognition memory test indicate that treadmill exercise pretreatments prevent decline in recognition memory in the VD rat model.

Next, we examined the potential mechanisms that might underline the treadmill exercise-induced improvement of recognition memory function in VD rats. It has been suggested that hippocampal structural synaptic plasticity constitutes the cellular basis of learning and memory, which requires the connections of synapses[35]. Our results showed decreased synapse numbers in the hippocamps of VD rats. In normal state, presynaptic terminal secretes memory-related substances via a canonical release machinery, while postsynaptic specialization senses substances via diverse receptors[36]. Therefore, the changes of synapse of synapse number are bound to affect synaptic structural plasticity. The study performed by Huang Y et al. on a murine model of vascular dementia by HE staining further indicate that significantly neuronal damage in the hippocampal of VD rat[37]. In this study, we also found that treadmill exercise led to an increase in the hippocampal synapse numbers of VD rats. Meanwhile, treadmill exercise increased the synapse numbers in the hippocampus in control group. The form and rearrange synapses under exercise is associated with enhancement of structural synaptic plasticity. Animal studies showed that exercise-induced enhancement of structural synaptic plasticity by regulating synaptic formation and rearrangement in normal states[38-39]. Thus, our study shed some light on the increase of synapse number by exercise that could be effective in exerting beneficial effects in VD rats.

Axons, dendrites, and dendritic spines constitute the structural basis of synaptic plasticity. The axon is functionally specialized to transmit signals, whereas the dendrites are specialized to receive signals[40]. In vivo imaging by FJB staining revealed that impaired of axonal and dendritic in the hippocampus after vascular dementia[41]. Dendritic spines are specialized postsynaptic structures that transduce presynaptic signals, are regulated by neural activity and correlated with learning and memory[42]. Our findings supported that the spine numbers of hippocampus were significantly decreased in the VD group compared to the control group. A review by Frankfurt M et al. reported that strong relationship between dendritic spine in the hippocampus and memory has been demonstrated in different spatial memory tasks[43]. Our study further revealed that treadmill exercise increased the spine numbers of hippocampus in VD rats.

It is likely that treadmill exercise pretreatment potentiates synaptic connections via an increase in dendritic spines under normal and dementia conditions[35, 44]. Such mechanisms might explain why treadmill exercise ameliorates the impairment of recognition memory in VD rat model.

In summary, treadmill exercise improved recognition memory, which can be contributed to the enhancement of hippocampal synapses number and dendritic spine density in VD rats. Our results collectively establish the central role of structural synaptic plasticity for neural network adaptations to exercises and provide more evidence for clinical intervention of memory deficits using exercise interventions.

Conclusions

Strengthening structural synaptic plasticity may represent a potential mechanism by which treadmill exercise

prevents decline in recognition memory and synapse loss in 2-VO induced VD rat model.

Declaration of conflicting interests

The authors declare that there is no conflict of interests.

Acknowledgement

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